

Application No.: 09/973,088
Amendment Dated 9 August 2004
Reply to Office Action of 8 April 2004

AMENDMENTS TO THE CLAIMS

This Listing of Claims will replace all prior versions, including listings, of claims in the application.

Listing of Claims

Claim 1 (currently amended): A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating pine cells of the *Pinus* subgenus with *Agrobacterium* for *Agrobacterium* transformation;

minimizing damage to cells subsequent to *Agrobacterium* infection by washing cells with a liquid wash culture medium, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and
germinating said transgenic somatic embryos to produce transgenic plants.

2 (currently amended): The method of claim 1, wherein said damage to cells is minimized by:

(a) suspending cells having been incubated with *Agrobacterium* in a liquid wash culture medium;

(b) agitating said liquid wash culture medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

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3 (previously presented): The method of claim 2, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

4 (currently amended): The method of claim 1, wherein said damage to cells is minimized by:

- (a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;
- (b) rinsing said cells using a liquid wash culture medium to remove *Agrobacterium*; and
- (c) recovering washed cells with minimal damage.

5 (previously presented): The method of claim 4, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

6 (previously presented): The method of claim 4, wherein pine cells are plated onto a support membrane subsequent to *Agrobacterium* transformation.

Claim 7 (original): The method of claim 4, wherein steps (b) and (c) are repeated until *Agrobacterium* contamination is no longer detectable.

Claim 8 (original): The method of claim 7, wherein said steps are repeated between 2 and 10 times.

9 (currently amended): The method of claim 4, wherein each wash is carried out for a duration sufficient to expose all the cells to the liquid wash culture medium, said wash carried out for between half an hour to overnight in duration.

Claim 10 (canceled).

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Claim 11 (original): The method of claim 4, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 12 (previously presented): The method of claim 1, wherein said selection is performed by

culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;
contacting said cells with a selection agent; and
selecting transformed cells.

Claim 13 (original): The method of claim 12, wherein said selection agent is contained in said gel medium.

Claim 14 (original): The method of claim 12, wherein said selection agent is contained in a layer and said support membrane is placed over said layer which is placed on said gel medium.

Claim 15 (previously presented): The method of claim 14, wherein said layer is a layer of liquid medium.

Claim 16 (previously presented): The method of claim 14, wherein said layer is a layer of gelled medium.

Claim 17 (original): The method of claim 14, wherein said layer is a filter paper with a liquid medium absorbed therein.

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Claim 18 (original): The method of claim 12, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 19 (previously presented): The method of claim 1 which further comprises the eradication of *Agrobacterium* from the pine cells after incubation with *Agrobacterium*.

Claim 20 (previously presented): The method of claim 19, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

Claim 21 (previously presented): The method of claim 20, wherein said layer is a layer of liquid medium.

Claim 22 (previously presented): The method of claim 20, wherein said layer is a layer of gelled medium.

Claim 23 (original): The method of claim 20, wherein said layer is a filter paper with a liquid medium absorbed therein.

Claim 24 (original): The method of claim 20, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

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Claim 25 (currently amended): A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating pine cells of the subgenus *Pinus* with *Agrobacterium* for *Agrobacterium* transformation;

eradicating *Agrobacterium* from the pine cells after incubation with *Agrobacterium*;

minimizing damage to cells subsequent to *Agrobacterium* infection by washing cells with a liquid wash culture medium, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

Claim 26 (currently amended): The method of claim 25, wherein said damage to cells is minimized by:

(a) suspending cells having been incubated with *Agrobacterium* in a liquid wash culture medium;

(b) agitating said liquid wash culture medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

Claim 27 (previously presented): The method of claim 26, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

Claim 28 (previously presented): The method of claim 26, wherein said selection is performed by

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culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;
contacting said cells with a selection agent; and
selecting transformed cells.

Claim 29 (previously presented): The method of claim 26, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

Claim 30 (previously presented): The method of claim 28, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

Claim 31 (currently amended): The method of claim 25, wherein said damage to cells is minimized by:

- (a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;
- (b) rinsing said cells using a liquid wash culture medium to remove *Agrobacterium*; and
- (c) recovering washed cells with minimal damage.

Claim 32 (previously presented): The method of claim 31, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

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Claim 33 (previously presented): The method of claim 31, wherein pine cells are plated onto a support membrane subsequent to *Agrobacterium* transformation.

Claim 34 (previously presented): The method of claim 31, wherein said selection is performed by

culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and

Selecting transformed cells.

Claim 35 (previously presented): The method of claim 31, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and

recovering cells from which said *Agrobacterium* has been eradicated.

Claim 36 (previously presented): The method of claim 34, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and

recovering cells from which said *Agrobacterium* has been eradicated.

Claim 37 (previously presented): The method of claim 25, wherein said selection is performed by

culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;

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contacting said cells with a selection agent; and
selecting transformed cells.

Claim 38 (previously presented): The method of claim 25, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

Claim 39 (currently amended): A method for minimizing damage to transformed cells of pine of the genus *Pinus* subgenus *Pinus* following infection by *Agrobacterium* for *Agrobacterium* transformation which comprises:

- (a) washing transformed cells of the subgenus *Pinus* in a liquid wash culture medium;
- (b) plating said cells on a support membrane;
- (c) suspending said cells in a liquid wash culture medium; and
- (d) recovering washed cells with minimal physical damage.

Claim 40 (original): The method of claim 39, wherein (i) cells are plated onto a support membrane and (ii) said cells are transformed prior to step (a).

Claim 41 (original): The method of claim 39, wherein steps (b) and (c) are repeated until *Agrobacterium* contamination is no longer detectable.

Claim 42 (original): The method of claim 41, wherein said steps are repeated between 2 and 10 times.

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Claim 43 (currently amended): The method of claim 39 wherein each wash is carried out for a duration sufficient to expose all the cells to the liquid wash culture medium, said wash carried out for between half an hour to overnight in duration.

Claim 44 (canceled).

Claim 45 (original): The method of claim 39, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 46 (previously presented): A method for pine cell tissue culture which comprises culturing pine cells of the genus *Pinus* subgenus *Pinus* on a support membrane placed over a gel medium.

Claim 47 (previously presented): The method of claim 46, wherein said support membrane is placed over a layer containing one or more tissue culture medium constituents, said layer is positioned on said gel medium.

Claim 48 (original): The method of claim 46, wherein said cells are plated onto said support membrane prior to culturing.

Claim 49 (previously presented): The method of claim 47, wherein said layer is a layer of liquid medium.

Claim 50 (original): The method of claim 47, wherein said layer is a filter paper with a liquid medium absorbed therein.

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Claim 51 (original): The method of claim 46, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 52 (previously presented): A method for selecting transformed cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells of the *Pinus* subgenus subsequent to transformation on a support membrane placed over a gel medium;
contacting said cells with a selection agent; and
selecting transformed cells.

Claim 53 (original): The method of claim 52, wherein said selection agent is contained in said gel medium.

Claim 54 (original): The method of claim 52, wherein said selection agent is contained in a layer and said support membrane is placed over said layer which is positioned on said gel medium.

Claim 55 (previously presented): The method of claim 54, wherein said layer is a layer of liquid medium.

Claim 56 (original): The method of claim 54, wherein said layer is a filter paper with a liquid medium absorbed therein.

Claim 57 (original): The method of claim 52, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer.

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Claim 58 (previously presented): A method for eradicating *Agrobacterium* from cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells of the *Pinus* subgenus on a support membrane over a layer containing an eradicator, said layer positioned in or over a gel medium; and

recovering cells from which said *Agrobacterium* contaminant has been eradicated.

Claim 59 (previously presented): The method of claim 58, wherein said layer is a layer of liquid medium.

Claim 60 (previously presented): The method of claim 58, wherein said layer is a layer of gelled medium.

Claim 61 (original): The method of claim 58, wherein said layer is a filter paper with a liquid medium absorbed therein.

Claim 62 (original): The method of claim 58, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claims 63-81 (canceled).